PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
PA/KIST 99346		
International application No.	International filing date (*day/month/year*)	Priority Date (*day/month/year*)
PCT/KR 99/00414	30 July 1999 (30.07.99)	31 July 1998 (31.07.98)
International Patent Classification (IPC) or national classification and IPC		
IPC[7]: A61K 9/0107		
Applicant		
KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examination Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

- I ☐ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28 February 2000 (28.02.00)	27 September 2000 (27.09.00)
Name and mailing address of the IPEA/AT Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer Mosser Telephone No. 1/53424/437

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00414

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-34 , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☒ the claims:
~~pages _____ , as originally filed~~
~~pages _____ , as amended (together with any statement) under Article 19~~
pages 35-40, 44 , filed with the demand
pages 41-43 filed with the letter of 25 September 2000 (25.09.00).

☒ the drawings:
pages 1/25 – 25/25 , as originally filed
pages _____ , filed with the demand
pages _____ , ~~filed with the letter of~~ _____.

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____.

☐ the claims, Nos. _____.

☐ the drawings, sheets/fig _____.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as „originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO
Inventive step (IS)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO
Industrial applicability (IA)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

With regard to the new claims 58 and 68, dated 25 September 2000 (25.09.00), novelty, inventive step and industrial applicability are recognized for all claims.

Claims;

1. A lipid emulsion comprising: 2-30 % of non-triglyceride oil; 0.01-20 % of one or more emulsifiers including a cationic surfactant; and, water to 100 %.

2. A solid lipid nanoparticle comprising: 2-30 % of fat of triglycerides having 10-18 carbons in each hydrophobic tail or ethyl stearate; 0.01-20 % of one or more emulsifiers including a cationic surfactant; and, water to 100 %.

3. A lipid emulsion loaded with a drug comprising: 2-30 % of squalene or squalane; 0.01-20 % of one or more emulsifiers; 0.1-10 % lipophilic or amphiphilic drug; and, water to 100 %.

4. A solid lipid nanoparticle loaded with a drug comprising: 2-30 % of ethyl stearate; 0.01-20 % of one or more emulsifiers; 0.1-10 % lipophilic or amphiphilic drug; and water to 100 %.

5. A method of preparing a lipid emulsion comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20 % of one or more emulsifiers including a cationic emulsifier and b) a second step of preparing emulsion of said aqueous phase with 2-30 % of non-triglyceride oil.

6. A method of preparing a solid lipid nanoparticle comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20 % of one or more emulsifiers including a cationic emulsifier with water; and, b) a second step of mixing said aqueous phase with 2-30 % of fat of triglycerides having 10-18 carbons in each hydrophobic tail or ethyl stearate.

7. A method of preparing a lipid emulsion loaded with a drug comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20 % of one or more emulsifiers with water, b) a second step of preparing an oil phase by mixing 0.1-10 % of lipophilic or amphiphilic drug and 2-30 % of non-triglyceride oil; and, c) a third step of preparing drug-loaded emulsion by mixing the aqueous and oil phases prepared in the first and second steps, respectively.

8. A method of preparing a solid lipid nanoparticle loaded with a drug comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20 % of one or more emulsifiers; b) a second step of mixing 0.1-10 % of lipophilic or amphiphilic drug and 2-30 % of ethyl stearate; and, c) a third step of preparing the drug-loaded solid lipid nanoparticle by mixing the aqueous and oil phases prepared in first and second steps, respectively.

9. The emulsion according to claim 1, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

10. The emulsion according to claim 1, wherein the non-triglycerides of step a) is squalene or squalane.

11. The emulsion according to any of claims 1, 9 or 10, wherein the emulsifier further comprises a phospholipid or a non-ionic surfactant.

12. The emulsion according to claim 1, wherein the cationic surfactant is selected from the group consisting of, 1,2-dimyristoyl-3-trimethylammonium-propane
1,2-dipalmitoyl-3-trimethylammonium-propane,
1,2-distearoyl-3-trimethylammonium-propane,
1,2-dioleoyl-3-trimethylammonium-propane,
1,2-dimyristoyl-3-dimethylammonium-propane,
1,2-dipalmitoyl-3-dimethylammonium-propane,
1,2-dilauroyl-3-dimethylammonium-propane,
1,2-distearoyl-3-dimethylammonium-propane,
dimethyldioctadecylammonium chloride,
N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride,
1,2-dioleoyl-3-ethylphosphocholine, and other cationic phospholipid.

13. The emulsion according to any of claims 1, 9 or 10, further comprising glycerol or fusogenic peptides.

14. The emulsion according to claim 13, wherein the fusogenic peptide is

polyethylene glycol of MW. 500-1000 or HA gp 41.

15. The emulsion according to claim 9, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

16. The emulsion according to claim 11, wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diacetylenic phosphilipid and derivatives thereof and the non-ionic surface active agent is selected from the group consisting of poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyehtylene ethers.

17. The emulsion according to claim 11, wherein the emulsifier is selected from the group consisting of 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol and bile salt.

18. The solid lipid nanoparticle according to claim 2, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

19. The solid lipid nanoparticle according to claims 2, wherein the emulsifier further comprises a phospholipid or a non-ionic surfactant.

20. The solid lipid nanoparticle according to claim 2, wherein the cationic surfactant is selected from the group comprising of,
1,2-dimyristoyl-3-trimethylammonium-propane,
1,2-dipalmitoyl-3-trimethylammonium-propane,
1,2-distearoyl-3-trimethylammonium-propane,
1,2-dioleoyl-3-trimethylammonium-propane,
1,2-dimyristoyl-3-dimethylammonium-propane,
1,2-dipalmitoyl-3-dimethylammonium-propane,
1,2-dilauroyl-3-dimethylammonium-propane,
1,2-distearoyl-3-dimethylammonium-propane,
dimethyldioctadecylammonium chloride,

N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride, 1,2-dioleoyl-3-ethylphosphocholine, and other cationic phospholipid.

21. The solid lipid nanoparticle according to claim 2, further comprising of glycerol or fusogenic peptides.

22. The solid lipid nanoparticle according to claim 21, wherein the fusogenic peptide is polyethylene glycol of MW 500-1000 or HA gp 41.

23. The solid lipid nanoparticle according to claim 18, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

24. The solid lipid nanoparticle according to claim 19, wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diacetylenic phosphilipid and derivatives thereof and the non-ionic surface active agent is selected from the group consisting of a poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyehtylene ethers.

25. The solid lipid nanoparticle according to claim 2, wherein the emulsifier is selected from the group consisting of 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol and bile salt.

26. The emulsion loaded with a drug according to claim 3, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

27. The emulsion loaded with a drug according to claim 3, wherein the emulsifier further comprises a phospholipid and a non-ionic surfactant.

28. The emulsion loaded with a drug according to claim 27, wherein the emulsifier is a phospholipid.

29. The emulsion loaded with a drug according to claim 27, wherein the surfactant is selected from the group consisting of, cationic, neutral and anionic phospholipids.

30. The emulsion loaded with a drug according to claim 3, further comprising glycerol, fusogenic peptides or proteins.

31. The emulsion loaded with a drug according to claim 30, wherein the fusogenic peptide is polyethylene glycol of MW 500-1000 or HA gp 41.

32. The emulsion loaded with a drug according to claim 26, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

33. The emulsion loaded with a drug according to claim 28, wherein the phospholipid is selected from a group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diacetylenic phosphilipid and derivatives and the non-ionic surface active agent is selected from a group comprising of a poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyehtylene ethers.

34. The emulsion loaded with a drug according to claim 3, wherein the emulsifier is selected from a group consisting of 1,2-dioleoyl-sn-3- phosphatidylethanolamine, diolein, fatty alcohol, cholesterol and bile salt.

35. The emulsion loaded with a drug according to claim 3, wherein the lipophilic or amphiphilic drug is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides,

immunosuppressants and immunostimulants.

36. The emulsion loaded with a drug according to claim 35, wherein the antiviral is diclofenac sodium or diclofenamic acid.

37. The emulsion loaded with a drug according to claim 35, wherein the immunosuppressant is cyclosporin A.

38. The emulsion loaded with a drug according to claim 3, wherein the lipid emulsion further comprises a hydrophilic drug in its aqueous phase.

39. The solid lipid nanoparticle loaded with a drug according to claim 4, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

40. The solid lipid nanoparticle loaded with a drug according to claim 4, wherein the emulsifier further comprises a phospholipid or a non-ionic surfactant.

41. The solid lipid nanoparticle loaded with a drug according to claim 40, wherein the emulsifier is a phospholipid.

42. The solid lipid nanoparticle loaded with a drug according to claim 41, wherein the phospholipid is selected from the group consisting of cationic, neutral and anionic phospholipid.

43. The solid lipid nanoparticle loaded with a drug according to claim 4, further comprising of a glycerol or fusogenic peptides or proteins.

44. The solid lipid nanoparticle loaded with a drug according to claim 43, wherein fusogenic peptide is polyethylene glycol of MW 500-1000 or HA gp 41.

45. The solid lipid nanoparticle loaded with a drug according to claim 39, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

46. The solid lipid nanoparticle loaded with a drug according to claim 41, wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diacetylenic phosphilipid and derivatives thereof and the non-ionic surface active agent is selected from the group consisting of a poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyehtylene ethers.

47. The solid lipid nanoparticle loaded with a drug according to claim 4, wherein the emulsifier is selected from the group consisting of 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol and bile salt.

48. The solid lipid nanoparticle loaded with a drug according to claim 4, wherein the lipophilic or amphiphilic drug is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants.

49. The solid lipid nanoparticle loaded with a drug according to claim 48, wherein the antiviral is diclofenac sodium or diclofenamic acid.

50. The solid lipid nanoparticle loaded with a drug according to claim 49, wherein the immunosuppressant is cyclosporin A.

51. The solid lipid nanoparticle loaded with a drug according to claim 4, wherein the solid lipid nanoparticle further comprises a hydrophilic drug in its aqueous phase.

52. A complex of the emulsion according to claim 1 and a biologically active material selected from the group consisting of DNA, ribonucleic acid,

antisense nucleic acid, ribosome, polynucleotide, oligonucleotide, and other pharmaceutical drugs.

53. The complex according to claim 52, further comprising glycolipid, lipopeptide, antibody, ligand for receptors or viral protein to target specific cells or organs.

54. The complex according to claims 52 or 53, further comprising protamine sulfate, histone or cationic polymer.

55. The complex according to claim 54, wherein cationic polymer is polylysine.

56. The complex according to claim 52, further comprising monovalent or multivalent salt.

57. The complex according to claims 53, wherein the cell is selected from the group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic cells, fetal cells, M cells, Langerhans islet cells, macrophages, plant cells, animal cells, and immotalized cell lines.

58. The complex according to claim 52, wherein the complex is transferred to cells via intravenous, intramuscular, intratracheal, intranasal, subcutaneous, parenteral or topical administration or through direct administration to a specific organ.

59. A method of using the emulsion according to claim 1 to deliver DNA or biologically active materials to target cells.

60. The complex according to claim 52, further comprising lipophilic or amphiphilic drug in an oil phase.

61. The complex according to claims 60, wherein the drug is an anticancer drug.

62. A complex of the solid lipid nanoparticle according to claim 2 and a biologically active material complex selected from the group consisting of DNA, ribonucleic acid, antisense nucleic acid, ribosome, polynucleotide, oligonucleotide, or other pharmaceutical drugs.

63. The complex according to claim 62, further comprising glycolipid, lipopeptide, antibody, ligand for receptors or viral protein to target specific cells or organs.

64. The complex according to claims 62 or 63, further comprising protamine sulfate, histone or cationic polymer.

65. The complex according to claims 64, wherein the cationic polymer is polylysine.

66. The complex according to claims 62, further comprising monovalent or multivalent salt.

67. The complex according to claims 63, wherein the cell is selected from the group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic cells, fetal cells, M cells, Langerhans islet cells, macrophages, plant cells, animal cells, and immotalized cell lines.

68. The complex according to claim 62, wherein the complex is transferred to cells via intravenous, intramuscular, intratracheal, intranasal, subcutaneous, parenteral or topical administration or through direct administration to a specific organ.

69. The complex according to claim 52, further comprising a lipophilic or amphiphilic drug in the fat.

70. The complex according to claims 69, wherein the drug is an anticancer drug.

71. The method according to claim 5, wherein the aqueous phase further comprises of 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

72. The method according to claim 6, wherein the aqueous phase further comprises of 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

73. The method according to claim 7, wherein the aqueous phase further comprises of 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

74. The method according to claim 8, wherein the aqueous phase further comprises of 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

75. The method according to claims 5, wherein the emulsifier is added in an oil phase instead of in an aqueous phase.

76. The method according to claims 6, wherein the emulsifier is added in a melted fat instead of in the aqueous phase.

77. The method of according to claims 7, wherein the emulsifier is added in the oil phase instead of in an aqueous phase.

78. The method according to claims 8, wherein the emulsifier is added in the melted fat instead of in the aqueous phase.